Prognostic impact of B-cell lymphoma 6 in primary CNS lymphoma.

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Abstract: BACKGROUND We investigated the prognostic significance of B-cell differentiation status and common B-cell differentiation markers in a post hoc analysis of 119 patients with primary CNS lymphoma (PCNSL) homogeneously receiving high-dose methotrexate (HDMTX)-based chemotherapy within the prospective G-PCNSL-SG1 trial. METHODS We evaluated protein expression of B-cell lymphoma 2 (BCL2), BCL6, CD10, and multiple myeloma oncogene 1/interferon regulatory factor 4 (MUM1/IRF4) by immunohistochemistry and analyzed the association with survival. RESULTS The median follow-up of all patients was 67.5 months. Median progression-free survival (PFS) was 10.61 months (95% CI: 4.23-17.00). Median overall survival (OS) was 28.85 months (95% CI: 17.96-39.73). Eighty-nine tumors expressed BCL2 (92.7%), 24 (20.5%) expressed CD10, 60 (54.1%) expressed BCL6, and 87 (79.0%) expressed MUM1/IRF4. On the basis of the Hans algorithm, 80 tumors (73.4%) were classified to the non-germinal center B group, suggesting a post-germinal center origin of PCNSL. Expression of BCL6 (cutoff point 30%), but none of the other markers, was associated with shorter PFS (P = .047) and OS (P = .035). On multivariate analysis, BCL6 expression was associated with shorter PFS (hazard ratio: 1.95, 95% CI: 1.22-3.12, P = .005) but not OS (hazard ratio: 1.85, 95% CI: 0.71-4.80, P = .21). Classification according to Hans algorithm and expression status of the single B-cell markers BCL2, CD10, and MUM1/IRF4 did not correlate with prognosis. CONCLUSION The findings are limited by the fact that only 23% of all G-PCNSL-SG1 patients could be included in the analysis. If validated in an independent cohort, BCL6 may assume clinical relevance as an unfavorable prognostic biomarker in PCNSL.

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Title:

Prognostic impact of BCL-6 in primary CNS lymphoma

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Running title: **Prognostic impact of BCL-6 in primary CNS lymphoma**

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Abstract

Background: We investigated the prognostic significance of B cell differentiation status and common B cell differentiation markers in a post hoc analysis of 119 patients with primary CNS lymphoma (PCNSL) homogenously treated with high-dose methotrexate (HDMTX)-based chemotherapy within the prospective G-PCNSL-SG1 trial. **Methods:** Protein expression of BCL-2, BCL-6, CD10 and MUM-1/IRF-4 was evaluated by immunohistochemistry, and the association with survival was analyzed. **Results:** The median follow-up of all patients was 67.5 months. The median progression-free survival (PFS) was 10.61 (95% CI 4.23-17.00) months; the median overall survival (OS) was 28.85 (95% CI 17.96-39.73) months. Eighty-nine tumors expressed BCL-2 (92.7%), 24 (20.5%) CD10, 60 (54.1%) BCL-6 and 87 (79.0%) MUM-1/IRF-4. On the basis of the Hans algorithm, 80 (73.4%) tumors were classified to the non-GCB group suggesting a post germinal center origin of PCNSL. BCL-6 expression (cut-off point 30%), but none of the other markers, was associated with shorter PFS (P=0.047) and OS (P=0.035). On multivariate analysis BCL-6 expression was associated with shorter PFS (HR 1.95, 95% CI 1.22-3.12, P=0.005) but not OS (HR 1.85, 95% CI 0.71-4.80, P=0.21). Classification according to Hans algorithm and expression status of single B cell markers BCL-2, CD10 and MUM-1/IRF-4 did not correlate with prognosis. **Conclusion:** The findings are limited by the fact that only 23% of all G-PCNSL-SG1 patients could be included in the analysis. If validated in an independent cohort, BCL-6 may assume clinical relevance as an unfavorable prognostic biomarker in PCNSL.

Introduction

Primary CNS lymphoma (PCNSL) is a rare aggressive B cell neoplasia most frequently of diffuse large B cell lymphoma type (DLBCL) that is confined to the CNS at time of diagnosis[1]. The formerly poor prognosis of PCNSL has been greatly improved by systemic high-dose methotrexate (HDMTX)-based polychemotherapy which provides the current standard treatment for all patients with PCNSL. Despite substantial improvements in the treatment of PCNSL the response to HDMTX-based chemotherapy is quite heterogeneous and overall prognosis remains poor with a median progression-free survival (PFS) of approximately 12 months and a median overall survival (OS) of about 3 years in most studies. This necessitates the identification and evaluation of reliable predictive and prognostic biomarkers for risk-stratified treatment decisions[2].

In systemic DLBCL, prognostic value of different biologic markers has widely been studied. Moreover, at least two prognostically important subgroups (germinal center B cell-like [GCB] and activated B cell-like [ABC] DLBCL) were characterized by gene expression profiling (GEP) using cDNA microarray[3,4]. Due to impracticability to perform microarray analysis on every patient, various immunohistochemical algorithms have been subsequently developed to capture the molecular diversity and stratify patients according to survival[5-8]. The most widely used method is the Hans algorithm separating DLBCL into germinal-center B cell (GCB) and non-germinal-center B cell (non-GCB) groups by using antibodies against CD10, BCL-6 and MUM-1/IRF-4[9]. In PCNSL, a few small and mostly retrospective studies either failed to observe a prognostic impact of common B cell differentiation markers or revealed contradictory results[10-14]. Here, we present the analysis of a set of B cell differentiation markers and their prognostic impact in a cohort from G-PCNSL-SG-1, the largest trial ever conducted in PCNSL.

Materials and Methods:

Patients and treatment

Immunocompetent adult patients with newly diagnosed PCNSL included in the G-PCNSL-SG1 trial with tumor samples available at one of the two reference pathology centers and tumor amount sufficient for immunohistochemical analysis were included. The inclusion and exclusion criteria were reported previously[15]. Patients were randomly allocated to receive first-line HDMTX-based chemotherapy with or without subsequent whole-brain radiotherapy (WBRT). Between May 2000 and August 2006, study therapy consisted of HDMTX (4 g/m^2 as a 4-h i.v. infusion with dose reduction according to creatinine clearence) on day 1 of six 14-day cycles; thereafter, patients were to receive HDMTX plus ifosfamide (1.5 g/m^2) on days 3–5 of six 14-day cycles. In those assigned to receive first-line chemotherapy followed by radiotherapy, WBRT was to be given at a total dose of 45 Gy in 1.5 Gy fractions. Patients allocated to first-line chemotherapy without WBRT who had not achieved complete response (CR) to HDMTX-based chemotherapy were given high-dose cytarabine (HDAraC; 2 × 3 g/m^2 on days 1–2 of 22-day cycles).

The study protocol was approved by local institutional review boards or ethics committees. All participants gave written informed consent.

Immunohistochemical analysis

Immunostaining on paraffin sections was performed centrally at two study reference pathology centers for CD10 (clone 56C6, Leica Biosystems, Newcastle upon Tyne, UK), BCL-6 (clone LN22, Leica Biosystems; clone PG-B6p, Dako, Hamburg, Germany), BCL-2 (clone100/D5 Leica Biosystems; clone 124, Dako) and MUM-1/IRF-4 (clone MUM1p, DAKO) using an automated immunostainer (BondMax™, Leica Biosystems; Benchmark XT, Roche-Ventana, Mannheim, Germany). Antigen retrieval was performed using appropriate

conditions, bound antibodies were made visible employing Bond Polymer Refine DAB, or ultraView Universal DAB detection kit (Roche-Ventana), developed and counterstained using the manufacturer´s protocols and reagents.

Immunohistological labeling of BCL-2, CD10, BCL-6 and MUM-1/IRF-4 was visually analyzed and interpreted according to the methods and cut-off points previously published by Horn *et al.*[16]. For BCL-6 staining, tumors with more than 30% positively labeled cells were considered positive. Tumors were further classified to GCB and non-GCB subgroups according to their expression of CD10, BCL-6 and MUM-1/IRF-4 using the methods and cut-off points (>30%) published by Hans *et al.*[9]. Tumors with CD10 expression, or BCL-6 expression without expression of MUM-1/IRF-4 were defined as GCB. The remaining tumors were classified as non-GCB.

Statistics

Progression-free survival (PFS) was defined as the time from study entry to first progression or death from any cause. Overall survival (OS) was defined as the time from study entry to death. PFS and OS were estimated by the Kaplan–Meier method. Group comparisons were carried out using the log-rank test. Additionally, simple and multiple Cox proportional hazard regression models were calculated, hazard ratios including two-sided 95% limits of confidence were calculated. The variables included in the multiple Cox models were determined by forward and backward variable selection leading to identical models. Non-significant variables were not included in the final models. Distribution of patients' characteristics to different groups was analyzed by the chi-square test. Mean values of independent groups were compared with Student's t-test. The level of significance was 0.05 (two-sided). Commercially available software was used (SPSS for Window, release 21.0).

Results:

Patient characteristics and clinical outcome

As central collection of tumor tissue was not intended at time of study enrolment, all available tumor material from participating centers were collected. Of 526 eligible patients treated within the G-PCNSL-SG1 trial, sufficient tumor material of 119 patients were available for the present immunohistochemical analysis. The main patient characteristics, treatment and outcome are summarized in Table 1. With exception of multifocal brain involvement and type of initial surgery for PCNSL patient characteristics were comparable to those of study patients not included in this analysis (Supplementary Table 1). However, there was a non-significant trend to better PFS (P=0.057) and OS (P=0.056) in patients included in our IHC analyses.

The median follow-up of all patients studied here was 67.5 months. The median PFS was 10.6 months (95% CI 4.2-17.00), the median OS was 28.9 months (95% CI 18-39.7).

Immunohistochemical profiles

Eighty-nine of 96 tumors (92.7%) expressed BCL-2 and 24 of 117 (20.5%) CD10. BCL-6 and MUM-1/IRF-4 were expressed in 60 of 111 (54.1%) and 87 of 110 (79.0%) tumors, respectively. Fifty-three tumors of the BCL-6-positive tumors co-expressed MUM-1/IRF-4 (88.3%). Twenty-nine tumors (26.6%) were classified as GCB and 80 (73.4%) as non-GCB group.

Prognostic analysis

Among the biologic markers, only BCL-6 expression correlated with shorter PFS and OS: *P*=0.047 and *P*=0.035, respectively (Fig. 1). On univariate analysis, the association of BCL-6

was significant for both PFS (HR 1.53, *P*=0.047, 95% CI 1.01-2.34) and OS (HR 1.66, *P*=0.035, 95% CI 1.04-2.65). On multivariate analysis, however, a significant association with PFS only was found: HR 1.95, *P*=0.005, 95% CI 1.22-3.12 (Table 2). All other biologic markers tested did not correlate with outcome. Particularly, classification according to the Hans algorithm revealed no significant difference between GCB and non-GCB subgroups with respect to survival outcome.

Patient characteristics and response to treatment did not differ between BCL-6-positive and -negative patients except for number of brain lesions (Supplementary Table 2) with BCL-6 expression significantly being associated with multifocal brain involvement (BCL-6-positive: 33.3% vs BCL-6-negative: 15.7%).

When the impact of commonly accepted clinical risk factors in PCNSL was tested, a significant association of age (HR 1.027, *P*=0.026, 95% CI 1.003-1.05) and Karnofsky Performance score (KPS) (HR 0.99, *P*=0.044, 95% CI 0.97-1.00) with OS was found on univariate analysis. The MSKCC score, which is deduced from age and KPS, showed a prognostic association with both PFS and OS in multivariate analyses (PFS: HR 1.87, *P*=0.011, 95% CI 1.15-3.04; OS: HR 2.95, *P*=0.016, 95% CI 1.22-7.13). Multifocal brain involvement was significantly associated with shorter PFS (HR 1.79, *P*=0.011, 95% CI 1.14-2.80) on univariate and shorter OS (HR 2.72, *P*=0.019, 95% CI 1.18-6.28) on multivariate analysis. To exclude that the effect of multifocal disease has been due to confounding with the type of surgery we performed adjusted and stratified analysis for these two factors for OS and PFS. In both adjusted analyses, multifocal disease remained significant after inclusion of type of surgery: PFS odds ratio = 1.78, *P*=0.012; OS odds ratio = 2.77, *P*=0.012. Within cases with resection, the odds ratio for PFS was 2.06; *P*= 0.043 and for OS 2.13; *P*=0.23; within cases with biopsy the odds ratio for PFS was 1.58; *P*= 0.131 and for OS 2.97; *P*=0.044 (the lack of significance in some subgroups was most probably due to their small size).

Discussion

Our data confirm previous studies indicating an activated B cell like immunophenotype and post-GC origin of most PCNSL[12,17-19] with frequent expression of the activation marker MUM-1/IRF-4, infrequent CD10 expression and expression of BCL-6, a marker of germinal center, in approx. half of the cases. In normal B cells, BCL-6 and MUM-1/IRF-4 are exclusively expressed[20] and high percentage of BCL-6/MUM-1/IRF-4 co-expression has been shown to be a characteristic feature of PCNSL as compared to systemic DLBCL[9,12,21-23]. Confirming previous analyses, a vast majority of our BCL-6-positive tumors co-expressed MUM-1/IRF-4 which indicates the activated immunophenotype of PCNSL[12,21]. Additionally, most PCNSL of our series belonged to the non-GCB group on the basis of the Hans algorithm, which is in accordance with earlier immunohistochemical analyses[12,17-19] and GEP analysis of 21 PCNSL cases[24].

The prognostic utility of B cell differentiation status and various B cell differentiation markers to predict outcome in PCNSL patients is currently questionable. The available data concerning this issue are based on mostly retrospective studies with small patient numbers and heterogeneous treatment schedules[10-14,18,19,21,25,26]. We here investigated expression and prognostic significance of B cell differentiation markers in the so far largest cohort of PCNSL patients homogenously treated with HDMTX-based chemotherapy within the prospective G-PCNSL-SG1 trial. BCL-2 as an antiapoptotic protein and normally down-regulated in germinal center B cells has been shown to be an independent poor prognostic indicator for systemic DLBCL[27,28]. In our series, we did not observe a correlation of BCL-2 expression with PFS or OS. Considering the low frequency of BCL-2-negative PCNSL, BCL-2 expression seems to be more a characteristic then a prognostic feature in PCNSL. The expression status of single B cell markers CD10 and MUM-1/IRF-4 also did not correlate with prognosis which is in accordance with previous analyses by Braaten *et al.* and

others[12,14,26]. However, the percentage of CD10-positive tumors might have been too low increasing the potential for confounding variables.

Previous analyses of the prognostic significance of BCL-6 expression in PCNSL yielded controversial results. Lossos *et al*. reported an independent positive association with PFS (and nearly significant with OS), and Braaten *et al*. found an independent and significant positive association with OS[13,26]. Both were retrospective studies with heterogeneously treated patients. Moreover, an unusually low positivity threshold of only 10% was used by Braaten *et al.*, resulting in expression frequency of 79%. In contrast, Momota *et al.* found an independent negative association of BCL-6 expression with PFS[14]. In the only prospective multi center trial addressing this question, CALGB 50202, BCL6 expression significantly correlated with shorter survival[11]. Our findings confirm a negative correlation of BCL-6 expression with outcome in PCNSL. Reasons for discrepancy with other studies could be the retrospective nature of previous studies, few patient numbers with consequent limited statistical power and possibility of confounding variables, non-uniform treatment and different immunophenotype and classification methods among different studies. Interestingly, there seems to be a correlation between BCL-6 expression and negative clinical characteristics in PCNSL. Camilleri-Broet *et al*. found an association with higher age and worse performance status[12]. In the present analysis, a correlation between BCL-6 expression and multifocal brain involvement was found. Multifocal brain involvement and inferior survival outcome was a finding in the whole G-PCNSL-SG1 cohort[29], and was also reported by others[30].

In our series, we did not observe a significant prognostic difference between the GCB and non-GCB subgroups. This is in line with several smaller studies[12,14,18,25].

Our study has several limitations that have to be regarded. Although based on the randomized controlled G-PCNSL-SG1 trial, the presented data is a formerly unplanned post hoc analysis of only 23% of all G-PCNSL-SG1 patients with an - unintended - positive selection bias for

analyzed patients. The bias was due to selection of patients on basis of availability of large tumor amount which resulted in higher percentage of patients with <2 tumor lesions and consecutively subtotal or total tumor resection. These factors were associated with better outcome in the total G-PCNSL-SG1 study population[29] and might explain the non-significant, but potentially biologically important trend to a superior prognosis in patients included in our IHC analyses. It remains unclear how this bias might have impacted our findings. A further shortcoming of the study is the number of patients analyzed and the number of events (PFS: 99, OS: 83). Both, although high as compared to previous studies, might have been too low for some outcome differences to become significant, and the multivariate analysis has to be interpreted with caution. Even so the selection of variables was identical with forward and backward methods a change of values in a small number of patients might have led to different models. This may also explain the differences between univariate and multivariate analyses for several factors which showed substantially higher odds ratios in multivariate as compared to univariate analysis (e.g. MSKCC score and multifocal brain involvement in the analysis of OS) .

Most of our patients (74.7%) were treated with HDMTX alone which was the standard first-line treatment regimen at time of study initiation. The type of chemotherapy did not have a significant prognostic impact in our uni- and multivariate analyses, but the number of patients might have been to small. Thus, we cannot exclude that the survival difference between the BCL-6-positive and BCL-6-negative group within our investigation could have been due to the less effective treatment regimen and therefore might not be applicable for patients undergoing an intensified first-line treatment.

In conclusion, our findings confirm an activated B cell like immunophenotype and post-GC origin of PCNSL and indicate BCL-6 expression as a valuable biomarker for inferior

prognosis. In view of the fact that several previous studies reported contradicting results, further prospective studies are necessary to validate our results.

References

1. Korfel A, Schlegel U. Diagnosis and treatment of primary CNS lymphoma. *Nat Rev Neurol.* 2013; 9(6):317-327.

2. Abrey LE, Yahalom J, DeAngelis LM. Treatment for primary CNS lymphoma: the next step. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2000; 18(17):3144-3150.

3. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature.* 2000; 403(6769):503-511.

4. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med.* 2002; 346(25):1937-1947.

5. Choi WW, Weisenburger DD, Greiner TC, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. *Clin Cancer Res.* 2009; 15(17):5494-5502.

6. Muris JJ, Meijer CJ, Vos W, et al. Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. *J Pathol.* 2006; 208(5):714-723.

7. Nyman H, Jerkeman M, Karjalainen-Lindsberg ML, Banham AH, Leppa S. Prognostic impact of activated B-cell focused classification in diffuse large B-cell lymphoma patients treated with R-CHOP. *Mod Pathol.* 2009; 22(8):1094-1101.

8. Meyer PN, Fu K, Greiner TC, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2011; 29(2):200-207.

9. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood.* 2004; 103(1):275-282.

10. Song MK, Chung JS, Joo YD, et al. Clinical importance of Bcl-6-positive non-deep-site involvement in non-HIV-related primary central nervous system diffuse large B-cell lymphoma. *J Neurooncol.* 2011; 104(3):825-831.

11. Rubenstein JL, Hsi ED, Johnson JL, et al. Intensive chemotherapy and immunotherapy in patients with newly diagnosed primary CNS lymphoma: CALGB 50202 (Alliance 50202). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2013; 31(25):3061-3068.

12. Camilleri-Broet S, Criniere E, Broet P, et al. A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. *Blood.* 2006; 107(1):190-196.

13. Lossos C, Bayraktar S, Weinzierl E, et al. LMO2 and BCL6 are associated with improved survival in primary central nervous system lymphoma. *Br J Haematol.* 2014; 165(5):640-648.

14. Momota H, Narita Y, Maeshima AM, et al. Prognostic value of immunohistochemical profile and response to high-dose methotrexate therapy in primary CNS lymphoma. *J Neurooncol.* 2010; 98(3):341-348.

15. Thiel E, Korfel A, Martus P, et al. High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. *Lancet Oncol.* 2010; 11(11):1036-1047.

16. Horn H, Ziepert M, Becher C, et al. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. *Blood.* 2013; 121(12):2253-2263.

17. Preusser M, Woehrer A, Koperek O, et al. Primary central nervous system lymphoma: a clinicopathological study of 75 cases. *Pathology.* 2010; 42(6):547-552.

18. Lin CH, Kuo KT, Chuang SS, et al. Comparison of the expression and prognostic significance of differentiation markers between diffuse large B-cell lymphoma of central nervous system origin and peripheral nodal origin. *Clin Cancer Res.* 2006; 12(4):1152-1156.

19. Levy O, Deangelis LM, Filippa DA, Panageas KS, Abrey LE. Bcl-6 predicts improved prognosis in primary central nervous system lymphoma. *Cancer.* 2008; 112(1):151-156.

20. Carbone A, Gloghini A, Larocca LM, et al. Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. *Blood.* 2001; 97(3):744-751.

21. Kinoshita M, Hashimoto N, Izumoto S, et al. Immunohistological profiling by B-cell differentiation status of primary central nervous system lymphoma treated by high-dose methotrexate chemotherapy. *J Neurooncol.* 2010; 99(1):95-101.

22. Chang CC, McClintock S, Cleveland RP, et al. Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. *Am J Surg Pathol.* 2004; 28(4):464-470.

23. Colomo L, Lopez-Guillermo A, Perales M, et al. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. *Blood.* 2003; 101(1):78-84.

24. Montesinos-Rongen M, Brunn A, Bentink S, et al. Gene expression profiling suggests primary central nervous system lymphomas to be derived from a late germinal center B cell. *Leukemia.* 2008; 22(2):400-405.

25. Raoux D, Duband S, Forest F, et al. Primary central nervous system lymphoma: immunohistochemical profile and prognostic significance. *Neuropathology.* 2010; 30(3):232-240.

26. Braaten KM, Betensky RA, de Leval L, et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. *Clin Cancer Res.* 2003; 9(3):1063-1069.

27. Mounier N, Briere J, Gisselbrecht C, et al. Rituximab plus CHOP (R-CHOP) overcomes bcl-2--associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). *Blood.* 2003; 101(11):4279-4284.

28. Gascoyne RD, Adomat SA, Krajewski S, et al. Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. *Blood.* 1997; 90(1):244-251.

29. Weller M, Martus P, Roth P, Thiel E, Korfel A. Surgery for primary CNS lymphoma? Challenging a paradigm. *Neuro Oncol.* 2012; 14(12):1481-1484.

30. Bessell EM, Graus F, Punt JA, et al. Primary non-Hodgkin's lymphoma of the CNS treated with BVAM or CHOD/BVAM chemotherapy before radiotherapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 1996; 14(3):945-954.

Figure 1:

Comparison of progression-free survival and overall survival according to BCL-6 expression.

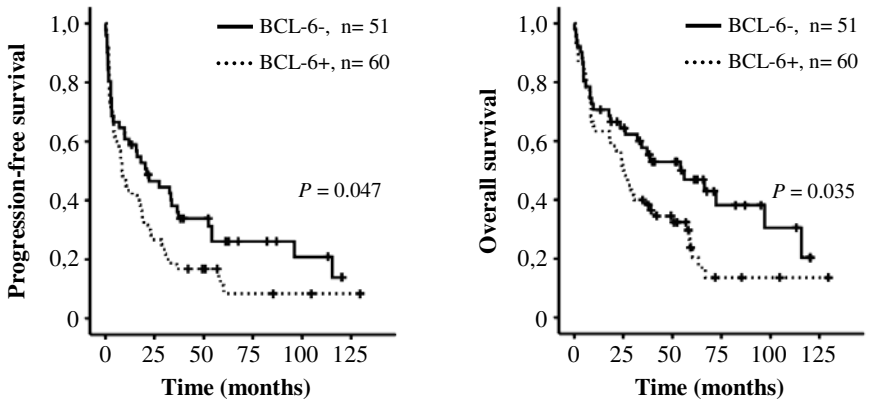

Table 1:

Patient characteristics, treatment and treatment response

Characteristics	All patients n=119 n (%)
Median age in years (range)	63 (26-83)
Males	62 (52.1%)
KPS, median	70
MSKCC score	
1	13 (10.9%)
2	59 (49.6%)
3	31 (26.1%)
No specification	16 (13.4%)
Serum LDH elevated	23 (19.3%)
No specification	59 (49.6%)
Meningeal involvement	6 (5.0%)
No specification	30 (25.2%)
No. of cerebral lesions	
0-1	82 (68.9%)
≥2 lesions	30 (25.2%)
No specification	7 (5.9%)
HDMTX versus HDMTX/IFO	89/30
WBRT	75 (63.0%)
Response to HDMTX-based chemotherapy	
Complete response	53 (44.5%)
Partial response	20 (16.8%)
Stable disease	6 (5.0%)
Progressive disease	27 (22.7%)
No specification	13 (10.9%)
Median PFS in months (95% CI)	10.61 (4.23-17.00)
Median OS in months (95% CI)	28.85 (17.96-39.73)

KPS, Karnofsky Performance Score; MSKCC, Memorial Sloan-Kettering Cancer Center; LDH, lactate dehydrogenase, HDMTX, high-dose methotrexate; IFO, ifosfamide; WBRT, whole-brain radiotherapy

Table 2: Univariate and multivariate analyses for PFS and OS

	Univariate analysis			Multivariate analysis		
	Hazard ratio	***P* value**	**95% CI**	**Hazard ratio**	***P* value**	**95% CI**
PFS						
Age[*]	1.01	0.32	0.99-1.03	n.d.	---	---
KPS[†]	0.99	0.23	0.98-1.01	n.d.	---	---
Gender[#]	0.81	0.29	0.54-1.20	0.69	0.108	0.44-1.08
Multifocal brain involvement[◊]	**1.79**	**0.011**	**1.14-2.80**	1.20	0.49	0.71-2.03
MSKCC score[+]	1.40	0.14	0.89-2.20	**1.87**	**0.011**	**1.15-3.04**
LDH[○]	1.27	0.43	0.70-2.28	1.41	0.32	0.72-2.76
Surgery (biopsy vs. resection)	1.28	0.24	0.85-1.91	0.94	0.80	0.57-1.54
HDMTX versus HDMTX/IFO	0.99	0.97	0.62-1.58	1.00	1.00	0.60-1.67
BCL-2[‡‡]	1.28	0.60	0.52-3.17	1.09	0.86	0.43-2.77
BCL-6[††]	**1.53**	**0.047**	**1.01-2.34**	**1.95**	**0.005**	**1.22-3.12**
CD10[##]	0.81	0.42	0.49-1.34	0.82	0.46	0.47-1.40
MUM-1/IRF-4[**]	0.96	0.89	0.58-1.60	0.77	0.37	0.43-1.37
GCB/non-GCB[++]	1.12	0.65	0.70-1.80	1.20	0.49	0.72-2.00
OS						
Age[*]	**1.027**	**0.026**	**1.003-1.05**	n.d.	---	---
KPS[†]	**0.99**	**0.044**	**0.97-1.00**	n.d.	---	---
Gender[#]	0.75	0.20	0.49-1.16	0.48	0.091	0.20-1.13
Multifocal brain involvement[◊]	1.47	0.11	0.91-2.39	**2.72**	**0.019**	**1.18-6.28**
MSKCC score[+]	**1.66**	**0.041**	**1.02-2.70**	**2.95**	**0.016**	**1.22-7.13**
LDH[○]	1.10	0.77	0.59-2.05	1.89	0.38	0.46-7.80
Surgery (biopsy vs. resection)	1.17	0.50	0.75-1.82	0.82	0.67	0.33-2.02
HDMTX versus HDMTX/IFO	0.92	0.76	0.53-1.60	0.37	0.19	0.084-1.63
BCL-2[‡‡]	0.94	0.89	0.38-2.33	0.68	0.62	0.16-3.01
BCL-6[††]	**1.66**	**0.035**	**1.04-2.65**	1.85	0.21	0.71-4.80
CD10[##]	0.61	0.09	0.35-1.07	1.00	1.00	0.39-2.57
MUM-1/IRF-4[**]	1.00	0.99	0.57-1.77	2.13	0.32	0.48-9.41
GCB/non-GCB[++]	1.46	0.17	0.86-2.48	1.14	0.79	0.44-2.98

[*]Age of 60 years or older versus younger than 60 years. [†]10% increase in Karnofsky Performance Score. In multivariate analyses, age and KPS were not separately but combined analysed as MSKCC score. [#]Female sex versus male sex. [◊]≥2 lesions versus 0-1 cerebral lesions. [+]MSKCC score 3 vs. 1/2. [○]Elevated serum lactate dehydrogenase (LDH) versus normal. [‡‡]BCL-2 positive versus negative expression. [††]BCL-6 positive versus negative expression. [##]CD10 positive versus negative expression. [**]MUM-1/IRF-4 positive versus negative expression. [++]GCB versus non-GCB according to Hans algorithm.

Supplementary Table 1:

Comparison of clinical characteristics, treatment and treatment response of patients with and without IHC analysis.

Characteristics	Patients with IHC analysis n=119 n (%)	Patients without IHC analysis n=405 n (%)	*P* value (with vs. without IHC analysis)
Median age in years (range)	63 (26-83)	63 (19-84)	0.34
Males	62 (52.1%)	236 (58.3%)	0.25
KPS, median	70	70	0.51
MSKCC score			
1	13 (10.9%)	66 (16.3%)	0.97
2	59 (49.6%)	165 (40.7%)	
3	31 (26.1%)	119 (29.4%)	
No specification	16 (13.4%)	55 (13.6%)	
Serum LDH elevated	23 (19.3%)	88 (21.7%)	0.09
No specification	59 (49.6%)	156 (38.5%)	
Meningeal involvement	6 (5.0%)	11 (2.7%)	0.22
No specification	30 (25.2%)	80 (19.8%)	
No. of cerebral lesions			
0-1	82 (68.9%)	190 (46.9%)	0.002
≥2 lesions	30 (25.2%)	145 (35.8%)	
No specification	7 (5.9%)	70 (17.3%)	
Type of surgical intervention			
Biopsy	43 (36.1%)	288 (71.1%)	<0.001
Subtotal resection	23 (23.2%)	47 (11.6%)	
Gross total resection	33 (27.7%)	33 (8.1%)	
No specification	20 (16.8%)	37 (9.1%)	
HDMTX versus HDMTX/IFO	89/30	311/94	0.71
WBRT	75 (63.0%)	202 (49.9%)	0.60
Response to HDMTX-based chemotherapy			
Complete response	53 (44.5%)	132 (32.5%)	0.111
Partial response	20 (16.8%)	88 (21.7%)	
Stable disease	6 (5.0%)	16 (4.0%)	
Progressive disease	27 (22.7%)	115 (28.4%)	
No specification	13 (10.9%)	54 (13.3%)	
Median PFS in months (95% CI)	10.61 (4.23-17.00)	6.08 (1.19-7.97)	0.057
Median OS in months (95% CI)	28.85 (17.96-39.73)	19.06 (14.87-23.24)	0.056

KPS, Karnofsky Performance Score; MSKCC, Memorial Sloan-Kettering Cancer Center; LDH, lactate dehydrogenase, HDMTX, high-dose methotrexate; IFO, ifosfamide; WBRT, whole-brain radiotherapy

Supplementary Table 2:

Comparison of clinical characteristics and outcome of patients according to BCL-6 expression status (n=111).

Characteristics	BCL-6+ (n=60)	BCL-6- (n=51)	*P* value (BCL-6+ vs. BCL-6-)
Age (years) Median (range)	65.5 (37-81)	63 (26-83)	0.61
Males	27 (45.0%)	30 (58.8%)	0.183
KPS, median	70	70	0.97
MSKCC score			
1	5 (8.3%)	6 (11.8%)	0.73
2	32 (53.3%)	23 (45.1%)	
3	17 (28.3%)	13 (25.5%)	
No specification	6 (10.0%)	9 (17.6%)	
Serum LDH elevated	11 (18.3%)	9 (17.6%)	0.98
No specification	30 (50.0%)	25 (49.0%)	
Meningeal involvement	2 (3.3%)	4 (7.8%)	0.40
No specification	15 (25.0%)	14 (27.4%)	
No. of cerebral lesions			
0-1	**37 (61.6%)**	**40 (78.4%)**	**0.046**
≥2 lesions	**20 (33.3%)**	**8 (15.7%)**	
No specification	**3 (5.0%)**	**3 (5.9%)**	
Type of surgical intervention			
Biopsy	33 (55.0%)	23 (45.1%)	0.47
Subtotal resection	11 (18.3%)	9 (17.6%)	
Gross total resection	15 (25.0%)	18 (35.3%)	
No specification	1 (1.7%)	1 (2.0%)	
HDMTX versus HDMTX/IFO	48/12	34/17	0.132
WBRT	41 (68.3%)	30 (58.8%)	0.33
Response to HDMTX-based chemotherapy			
Complete response	26 (43.3%)	26 (51.0%)	0.79
Partial response	10 (16.7%)	7 (13.7%)	
Stable disease	4 (6.7%)	2 (3.9%)	
Progressive disease	14 (23.3%)	10 (19.6%)	
No specification	6 (10.0%)	6 (11.8%)	
Median PFS (months)	**8.48 (5.07-11.89)**	**20.96 (8.03-33.92)**	**0.047**
Median OS (months)	**24.87 (20.71-29.03)**	**54.11 (19.71-88.51)**	**0.035**

KPS, Karnofsky Performance Score; MSKCC, Memorial Sloan-Kettering Cancer Center; LDH, lactate dehydrogenase, HDMTX, high-dose methotrexate; IFO, ifosfamide; WBRT, whole-brain radiotherapy